INTRODUCTION

New strategies to improve clinical response and prevent side effects of cancer therapy focused on substances that can be used with usual treatment. In particular, new tumor-targeted agents focus on cell cycle arrest, apoptosis or premature cell-cycle checkpoint exit. This study evaluates the effect of a combined treatment with curcumin or oxaliplatin and curcumin, on cell viability, cell cycle parameters and apoptosis in human ovarian cancer cell line (2008) and in cisplatin-resistant variant (C13). Principal drugs used in epithelial, hepatic and ovarian cancer treatment are curcumin and oxaliplatin. They act at nuclear level of cells, after non-antagonistic conversion to active derivatives, forming platinum-DNA adducts and DNA-protein cross links. Moreover, they indirectly cause apoptosis and cell-cycle perturbation, by blocking DNA replication and transactivation. For this, they are characterized by many side effects, such as toxicity and an intrinsic and acquired resistance. To reduce side effect we decided to combine platinum drugs with a cytotoxic agent: curcumin.

Curcumin (diferuloylmethane) isolated from rhizome of Curcuma longa, is a naturally occurring antioxidant and anti-inflammatory agent. Preclinical and clinical evidence support curcumin chemopreventive and antiinflammatory effects, because it acts into intracellular signaling pathways related to cell proliferation and apoptosis by affecting various molecular targets, such as up-regulating cell death and p53 and down-regulating cyclin D1, cdk-1, cdk-2 and NFκB.

The aim of the study was to evaluate the effect of a combined treatment with platinum drugs and curcumin, on cell viability, ROS generation, cell-cycle and apoptosis in human ovarian cancer cell line (2008) and in cisplatin-resistant variant (C13).

RESULTS

Results showed that curcumin and platinum drugs per se caused time (24-72h) and concentration-dependent (1-100μM) reduction in cell viability, which persisted after 72h (Fig. 1). Moreover, we noticed that curcumin is more active in cisplatin-resistant cells than in wild type ones. Combination of curcumin and platinum drugs had a synergetic effect both in wild type 2008 and c13 cells (results not shown).

As regards to the cell-cycle, this is significantly different in untreated wild type and resistant cells, but curcumin reduced the percentage of cells in G2/M phase and caused apoptosis increase sub-G0, both in 2008 and c13 cells. Moreover, curcumin increased number of c13 cells in G2/G1 phase and reduced cells percentage in S (Fig. 2). In wild type cells and resistant ones, after a combination treatment curcumin (3.5-1.5 μM) and platinum drugs, we observed an increased apoptosis and G0/G1 phase (not in resistant cells), and a G2/M decreased in comparison with drugs alone (Fig. 2).

We decided to test also reactive oxygen species (ROS) generation and glutathione levels on cancer cells after treatment with curcumin, to test the antioxidant action of the one. ROS generation was fast after treatment with curcumin (0.5-1.5 μM). We measured a lowest basal ROS level in cisplatin-resistant cells (compared to wild type). In both cells lines (Fig. 3), intracellular glutathione (GSH) was measured after 2h and 24h of treatment with curcumin (0.5-1.5 μM). Basal level of GSH were higher in resistant cells than in wild type. In both lines, curcumin decreased level of GSH after 2h, whereas it increased GSH after 24h. Moreover, it increased intracellular level both in C13 and 2008 cells, suggesting that the increased susceptibility of ovarian cancer cell to combined treatment is not related to a glutathione depletion.

CONCLUSIONS

Our results suggest that curcumin inhibits cell viability of ovarian cancer cells. Furthermore, compared to single-drug treatment, curcumin combined with cisplatin and oxaliplatin drugs, of concentration lower than C50, causes best dose- and time-dependent increases in cell-cycle arrest and apoptosis. The combination makes cisplatin-resistant cell sensitive to the drug, a further suggested mechanism for drug resistance is increased in intracellular thios in the redox pathway, which may Inactivate and remove platinum compounds.

Our experiments show that in cisplatin-resistant cells [compared to cisplatin-sensitive ones], higher GSH content is parallel to lower ROS level, and that curcumin causes an increase of ROS after 2h incubation, but increase in GSH after 24h. So curcumin has early pro-apoptotic and late antioxidant effect. In conclusion, increased glutathione level in both C13 and 2008 cells, suggesting that the increased susceptibility of ovarian cancer cell to combined treatment is not related to a glutathione depletion. In our study, we demonstrated that curcumin is cytotoxic in ovarian cancer cells and that it increases effect of platinum drugs. Our future purpose will be investigate into chemopreventive and chemopreventive properties of curcumin and its synergism with platinum drugs.

REFERENCES


Curcumin: The most active polyphenol derived from the plant Curcuma longa, commonly called turmeric. It is not only a naturally occurring antioxidant and anti-inflammatory agent but also it has chemopreventive and anti-inflammation effects, because it acts into intracellular signaling pathways related to cell proliferation and apoptosis and in this study we used a solution of curcumin at different concentrations, prepared in DMSO and used in cultured medium.

METHODS

Cell lines and reagents: 2008 and c13 cells derive from human ovary carcinoma. The cisplatin-resistant variant were obtained by in vitro selection following with increasing concentrations of cisplatin. Cells were grown in RPMI 1640 medium, 10% fetal bovine serum, 3% l-glutamine and 1% pen-strep, in humidified condition at 95% CO2 and 37°C.

All reagents were from Loraas (Basel, Switzerland).

Cell viability assay: was determined after 24h, by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) and XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) reagents, prepared in DMSO and used in enzymatic conversion to active derivatives, forming yellow and purple forms, respectively.

Cell-cycle analysis by flow cytometry: after 24h of treatment, cells were fixed and suspended in a solution of propidium iodide (100 μg/ml, Merck, Darmstadt, Germany) and RNaseA (100 μg/ml) free (sigma) in PBS for 30 min, at room temperature in the dark. Cells were analyzed in an Epics XL flow cytometer and a photomultiplier PMT2.

Annexin V/propidium iodide staining for apoptosis: cells were stained with Alexa 488/Annexin V-propidium iodide (PI) and analyzed on an Epics XL flow cytometer. Cell staining negative for both annexin V and PI are viable, cells annexin V+/PI+ are in early apoptosis, cells annexin V+/PI+ are necrotic or in late apoptosis.

ROS Intestinal level: detection of Reactive oxygen species (ROS) was carried out on living cells using 2’,7’-dichlorofluorescin-diacetate (Molecular Probes), after 2h of treatment. Cells were analyzed on an Epics XL flow cytometer.

Intracellular glutathione assay: Intracellular glutathione concentrations were measured by addition of 2 μl glutathione reductase on coulter cells. Product formation was recorded continuously with a spectrophotometer. Total amount of glutathione in samples was determined from a standard curve obtained by plotting known amounts (0.5-4 μg/ml) of glutathione against rate of change in absorbance at 412nm.

Curcumin and platinum drugs combination in human ovarian carcinoma cells: cell-cycle inhibition and apoptosis.

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